bio-protocol Preprint

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Gene-specific m6A qPCR

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An abbreviated version of this protocol was published in Science Advances in May 2021

Epitranscriptomic regulation of insecticide resistance

DOI: 10.1126/sciadv.abe5903

Detailed protocol

Thiamethoxam resistant and susceptible mRNAs were subject to Methylated RNA Immunoprecipitation (MeRIP) analysis using the Magna MeRIP m^6A kit (17-10499, Millipore) to profile of m^6A RNA methylation sites. CYP4C64-specific m^6A qPCR was used to determine m^6A abundance across transcripts of this gene. Briefly, 30µg of total RNA was sheared to about 100nt in length by metal-ion-induced fragmentation, then purified and incubated with anti-m^6A antibody-conjugated beads in 100µl 1×immunoprecipitation buffer supplemented with RNase inhibitors at 4 °C for 2 hours. Methylated RNA was immunoprecipitated with beads, eluted by competition with free m^6A, and recovered with elution buffer, and converted to cDNA (Taraka Biotech), further analysed by qPCR along with MeRIPed RNA (TIANGEN). The relative enrichment of m^6A in each sample was calculated by normalizing to a reference gene EF1α (RPL29 cannot determine in the MeRIPed RNA). Each group was repeated in triplicate.

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Bass, C. and Zhang, Y. (2021). Gene-specific m6A qPCR. Bio-protocol Preprint. bio-protocol.org/prep1069.
2. Yang, X., Wei, X., Yang, J., Du, T., Yin, C., Fu, B., Huang, M., Liang, J., Gong, P., Liu, S., Xie, W., Guo, Z., Wang, S., Wu, Q., Nauen, R., Zhou, X., Bass, C. and Zhang, Y.(2021). Epitranscriptomic regulation of insecticide resistance. Science Advances 7(19). DOI: 10.1126/sciadv.abe5903

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